

MICROBIAL SOURCE TRACKING in New Hampshire Coastal Waters Using *Escherichia coli* Ribotyping

Steve Jones

Center for Marine Biology/Jackson Estuarine Lab
University of New Hampshire

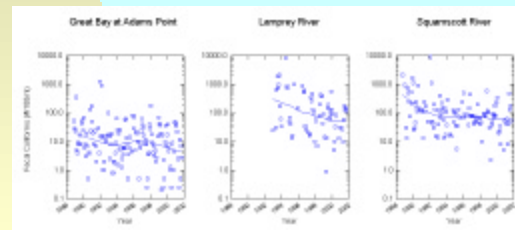
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INTRODUCTION

Water Quality Issues in the NH Seacoast

- § Recreational shellfish harvesting is limited in some NH Seacoast shellfish waters;
- § Studies have shown widespread pollution problems associated with storm events & sewage system infrastructure;
- § Remedial actions following shoreline surveys in some problem areas have not been successful in improving water quality.

Temporal trends for fecal coliforms in Great Bay (Langan & Jones)



Traditional Indicators for Identifying Fecal Sources

- Total & fecal coliforms, *Escherichia coli*, enterococci
 - not fecal specific,
 - poor correlation with GI illness,
 - don't survive as long as viruses in marine waters,

DO NOT differentiate animal from human sources when routine analyses performed

NEW TOOL: Microbial Source Tracking

IDENTIFY species that are fecal contamination
SOURCES, not just concentrations of bacteria

Ribotyping

- DNA profiling of bacterial strains;
- Target DNA (rRNA) conserved, yet variable enough to determine differences between isolates from different animals.
- Gaining acceptance for MST.
- Potential **significant savings of resources** in efforts to improve coastal water quality.

MATERIALS AND METHODS

Ribotyping: Lab Procedures

- § *E. coli* isolated and identification verified: source species & water samples.
- § DNA extracted & purified.
- § DNA digested w/restriction enzyme.
- § DNA separated via gel electrophoresis.
- § DNA denatured & blotted onto membrane.
- § Hybridization with *E. coli* rRNA DNA probe.
- § DNA exposed to a chemiluminescent substrate & digitally imaged.
- § Image enhanced & optimized in computer.

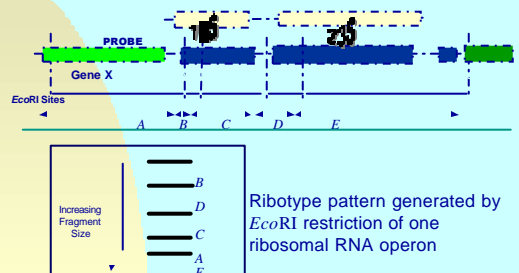
Ribotyping: RiboPrinter Procedures

- § *E. coli* isolated and identification verified: source species & water samples.
- § Load culture into RiboPrinter.
- § DNA banding profile images enhanced & optimized in computer.

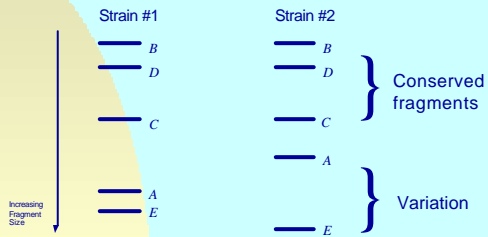
RiboPrinter® System - Universal Tool for the Micro Lab

- § Identification AND Characterization
- § Ease of Use
 - ⌘ Fully automated, minimal hands-on time
- § Speed
 - ⌘ Results in 8 hours
- § Accuracy
- § Standardization

Ribotyping (RiboPrinter): Example Using *EcoRI*



Species Differentiation with Ribotyping



same 16S & 23S+ sequence; different ribotype patterns

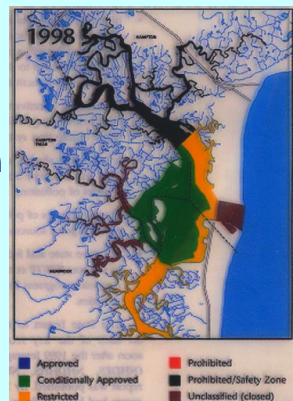
Source Identification: Data Analysis

- ⌘ DNA patterns are analyzed by computing similarity using Dice's coincidence index.
- ⌘ Source species identification for water sample patterns is based on degree of matching to source species patterns.

STUDY SITE DESCRIPTION

Shellfish water classification

Hampton/Seabrook Harbor



Hampton/Seabrook Harbor

- § Important soft shell clam recreational harvesting area.
- § Mostly sewerage, continued problems with bacterial contamination.
- § Need to identify contamination sources during small rainfall events and during all conditions in autumn.

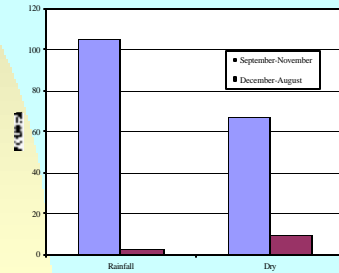
ROUTINE MONITORING

What are sources of contamination in harbor?

- Fecal coliforms used to classify shellfish-growing waters;
- Ten (10) sites for routine water sampling;
- Samples collected from 8/7/00 to 10/29/01.

RESULTS

Geometric mean fecal coliforms in Hampton Harbor (8/00-10/01) Seasonal and weather effects

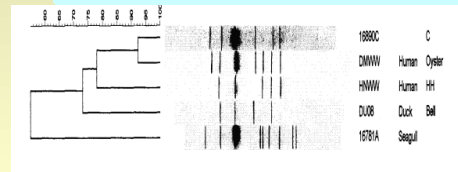


Source Species Database: “Manual Ribotyping

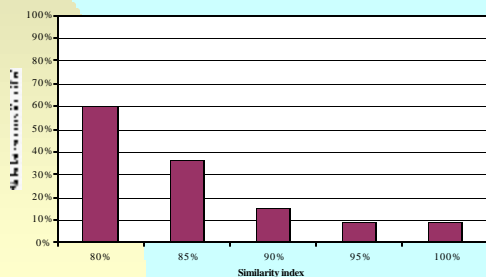
Source species category	Source species	# of isolates
HUMANS/WASTEWATER	wastewater	46
	humans	5
PETS	cat	2
	dog	9
DOMESTIC ANIMALS	chicken	2
	cow	32
	horse	14
WILD ANIMALS	covote	5
	Aw	43
	muskkrat	3
	raccoon	28
	red fox	24
AVIAN SPECIES	cormorant	15
	duck	4
	geese	19
	gull	5
	pigeon	2
	robin	3
	Total	261

Cluster analysis of ribotype gel banding patterns:

Human = 93% similarity to water sample isolate



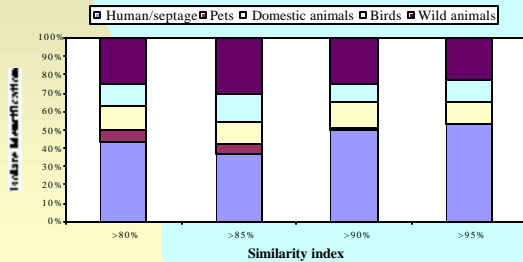
Hampton Harbor isolate source identification success at different similarity indexes



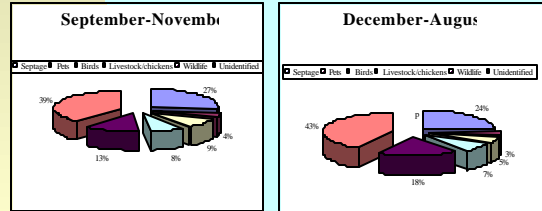
Source species for identified profiles --80% similarity--

Source species type	# of isolates	% in study area
Humans/septage	102	26%
Pets	15	4%
Birds	29	7%
Domestic animals	30	8%
Wild animals	59	15%
Unidentified	155	40%
	390	

Isolate identification at different similarity indexes:



Effect of Season on Source Species



CONCLUSIONS: Routine Monitoring

- § Humans are the most significant source species of fecal pollution in Hampton Harbor.
- § Wild animals as a group are an important type of source
- § Source species distributions were affected only slightly by rainfall and season.

STORMWATER PIPES

What is the significance of stormwater pipes as sources of fecal pollution?

- Two sites, one each in Seabrook and Hampton;
- Samples collected during a rainfall event (1.39"/12 h) on 10/16/02;
- Water samples collected during 5 stages of runoff hydrograph.

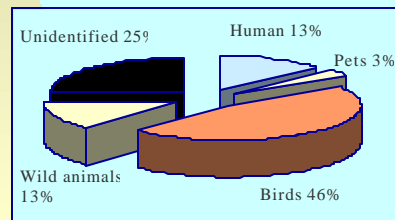
Source Species Database: RiboPrinter

Source species category	Source species	# of Isolates	Source species category	Source species	# of Isolates
"HUMANS"	septage	16	DOMESTIC ANIMALS	alpaca	3
	wastewater	107		buffalo	5
	humans	82		chicken	3
PETS	cat	21		cow	56
	dog	37		goat	4
AVIAN SPECIES				horse	28
				sheep	2
	coronant	12	WILD ANIMALS	293	
	duck	16		coyote	29
	geese	39		deer	93
	gull	28		mouse	12
	pigeon	5		muskrat	2
	robin	4		otter	14
	sparrow	3		raccoon	84
	starling	3		rabbit	27
	wild turkey	7		red fox	27
				skunk	5

TOTAL = 774

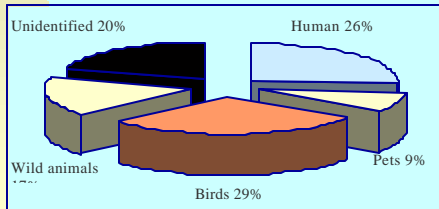
Source species (90% similarity) in storm water from pipe in Hampton, NH: 10/16/02

E. coli = 72,000 - 304,000 cfu/100 ml



Source species (90% similarity)
in storm water from pipe in Seabrook, NH:
10/16/02

E. coli = 14,400 - 1,120,000 cfu/100 ml



CONCLUSIONS: Hampton/Seabrook Harbor

- § Use of the RiboPrinter provided more accurate identification;
- § Birds were an important source type in both pieps, humans were more important in the Seabrook pipe;
- § The profiles of source species identified at the stormwater pipes were different from each other and from what was found in the Harbor.

Types of source species identified for *E. coli* isolates from NH oysters and overlying water

WATER AND TISSUE				
Source sp. type	APPROVED AREA		PROHIBITED AREA	
	Isolates	%	Isolates	%
Human	5	17%	9	24%
Pet	0	0%	2	5%
Bird	2	7%	3	8%
Wild animal	1	3%	1	3%
Livestock	17	57%	16	43%
Unknown	5	17%	6	16%
PROHIBITED AREA				
Source sp. type	OVERLYING WATER		OYSTER TISSUE	
	Isolates	%	Isolates	%
Human	5	26%	4	22%
Pet	1	5%	1	6%
Bird	1	5%	2	11%
Wild animal	1	5%	0	0%
Livestock	8	42%	8	44%
Unknown	3	16%	3	17%

Research Needs

- § A larger source species database;
- § Wild animal sources: Ground truth; Public health threat; Loading verification
- § Sampling design;
- § Reduce costs: modify study design.
- § Temporal and geographical effects on the consistency of intraspecies bacterial strains;
- § Methods comparison studies.

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